

STUDY ON THE EFFECT OF BIOLOGICAL FOLIAR AND SOIL FERTILIZERS AND CONVENTIONAL FERTILIZERS ON THE YIELD OF COMMON WINTER WHEAT

Elisaveta VASILEVA¹, Dilyana BLAGOVA², Evgeniy DIMITROV¹, Zlatina UHR¹

¹Agricultural Academy, Institute of Plant Genetic Resources “Konstantin Malkov”,
2 Druzhba Street, Sadovo, Bulgaria

²Profeco Ltd, 29 Nikola Petkov Street, Plovdiv, Bulgaria

Corresponding author email: genipd@abv.bg

Abstract

*The aim of the present study is to investigate the effects of biological soil improvers - liquid organic fertilizers obtained by different methods - through nanotechnology and through hydrolysis of plant meals - and to determine the optimal doses of these preparations for maximizing grain yields from new genotypes of common winter wheat (*Triticum aestivum*) developed by the method of combinatorial breeding. The experimental plot is established in the biological experimental field of the Institute of Plant Genetic Resources in Sadovo (Bulgaria), with three replications of seven fertilization treatments, over two consecutive growing seasons: 2023/2024 and 2024/2025. In the older hybrid wheat selections, grain yields are more strongly influenced by the type and quantity of applied fertilizers, as well as by agrometeorological conditions. The highest and most stable yields are obtained from the newest breeding lines. The effect of the fertilizer containing phytohormones depends primarily on agrometeorological conditions; when sufficient moisture is available, the active ingredients of the fertilizer are effectively absorbed, and increasing the dose leads to higher grain production. The effect of the nanofertilizer liquid humus on yields is weaker, limited at a level of 1000 ml/da, and the results of its use depend on the sink capacity of the genotype.*

Key words: *Triticum aestivum*, nanofertilizer, grain yield.

INTRODUCTION

Contemporary research shows that both biological fertilizers including organo-mineral and microbiological products and conventional mineral fertilizers have a positive effect on the yield of winter wheat, though with some differences in their effects depending on the growing conditions. Conventional fertilizers ensure the direct input of essential macro-nutrients such as nitrogen, phosphorus, and potassium, which are critically important for plant growth and productivity. Biological preparations containing effective microorganisms and humic acids improve soil structure, enhance microbiological activity, and increase nutrient uptake, which can result in greater plant resilience and more efficient nutrient utilization (Sydiakina & Dvoretzkyi, 2020; Dehodyuk & Muliarchuk, 2023; Ashurbekova et al., 2024). Nanofertilizers are a promising innovation for increasing grain yields, but they require precise application and integration into an overall fertilization strategy to achieve optimal results.

Studies have shown high values of plant cell viability when treated with nanoparticles, which indicates an absence of toxicity at appropriate concentrations. Nanofertilizers containing iron, molybdenum, and silicon dioxide improve physiological and biochemical plant parameters by enhancing growth and viability, and they support higher activity of soil enzymes, leading to increased yields and improved quality of wheat grain products (Gavrish et al., 2019; Ahmadian et al., 2021; Elahi et al., 2025).

Comparative field-trial data indicate that conventional farming systems provide higher grain yields compared to organic systems. Organic cultivation is a strategic choice based on key advantages for environmental protection and sustainable development. In organic agriculture, yields are lower but stable, and long-term increases in soil organic matter, improved soil structure, and enhanced biodiversity are observed, contributing to sustainable production of healthy food and maintaining agrosystem fertility under adverse conditions (Novokhatsky et al., 2019; Le

Campion et al., 2020; Zastavnyy, 2020; Vinyukov, 2023). Currently, no widely published systematic reviews or meta-analyses exist comparing the effectiveness of nanofertilizers and biofertilizers containing phytohormones in cereal crops, as this is a relatively new and rapidly developing field.

The aim of the present study is to investigate the effects of biological soil improvers - liquid organic fertilizers obtained by different methods, namely through nanotechnology and through hydrolysis of plant meals - and to determine the optimal doses of these preparations for maximizing grain yields from new *Triticum aestivum* genotypes developed through combinatorial breeding. The results contribute to the development of adaptive agroecosystems and the search for synergistic effects in integrated agrotechnologies.

MATERIALS AND METHODS

The biological materials used in the experiment include the wheat varieties Sadovo 1, Pobeda, and Avenyu, as well as the breeding lines Magizhi, MH 258/3355 and RU 251/268. The experimental plot was established in the biological experimental field of the Institute of Plant Genetic Resources in Sadovo (Bulgaria), with three replications of seven fertilization treatments, over two consecutive growing seasons: 2023/2024 and 2024/2025. The crop rotation involved a black fallow, and the soil type was Chromic Vertisol.

To compare the results, two controls were used: an untreated control in the biological field and a control grown under conventional technology (outside the biological field, in a stationary fertilizer experiment). In the conventional system, ammonium nitrate (NH_4NO_3) was applied at an equivalent of N_{12} , along with the herbicides Derby (florasulam 150 g/kg, aminopyralid 300 g/kg) and Axial (pinoxaden 50 g/l), and the insecticide Oasis (esfenvalerate 50 g/l).

The biological cultivation system included pre-sowing spraying with 300 ml/da *Bacillus megaterium* and two treatments with the fungicide Sulfeco (nano-sulfur) in March and April at a dose of 50 ml/da. The study examined the effects of the soil improvers Humeko (liquid humus obtained through nanotechnology) and

Lumbreko (liquid organic fertilizer obtained through hydrolysis of plant meals), which were applied by spraying in March at the following doses: Humeko: 500 ml/da, 1000 ml/da, 1500 ml/da; Lumbreko: 100 ml/da, 200 ml/da, 300 ml/da, and 400 ml/da. The data were subjected to variation and dispersion (ANOVA) analysis.

RESULTS AND DISCUSSIONS

The sowing period begins with drought in October, which is more pronounced in the second year of the experiment and delays both sowing and seed germination and emergence. High temperatures and late emergence lead to weaker root development and hardening, but during the winter months there are no conditions for winterkill. Stands with normal density, uniformity, and viable plants are formed. Vernalization conditions are present for two months during both growing seasons. Nutrient supply during the light stage and the organogenesis of the inflorescences is ensured, despite the uneven distribution of rainfall across phenophases. Plants with genotype-specific habitus are formed.

During the stem-elongation stage in the first growing year, drought is observed, whereas in the second year temperatures and soil moisture are favorable for accelerated biomass accumulation and for ensuring floret fertility. During the heading stage, which is critical in terms of water demand, temperatures and soil moisture favor rapid and uniform emergence and full development of the inflorescences. Pollination and grain formation occur under favorable assimilation conditions.

The processes of grain filling, milk ripeness, and waxing maturity are accelerated by the high temperatures ($+4^\circ\text{C}$ and $+3^\circ\text{C}$ above the long-term averages in the two consecutive years). In the first harvest year (2024), the phase is further shortened by water deficit, while in the second year good water supply to the plants is maintained due to soil moisture reserves. Conditions during the waxing maturity and full ripeness stages are favorable for the accumulation of reserve nutrients in the grain. Meteorological conditions in 2024/2025 are comparable to the long-term norms for the region, whereas the experimental year 2023/2024 is dry and hot (Table 1).

Table 1. Agrometeorological conditions

Month	1991-2020 r	2023/ 2024 r	Deviation	2024/2025 r	Deviation
Mean monthly temperature (T avg, °C)					
X	13.3	16.2	2.9	13.9	0.6
XI	7.4	9.0	1.6	5.5	-1.9
XII	2.2	4.7	2.5	5.5	3.3
I	0.9	3.8	2.9	3.0	2.1
II	3.1	8.9	5.8	2.1	-1.0
III	7.6	10.1	2.5	10.5	2.9
IV	13.0	16.0	3.6	13.0	0.0
V	17.9	20.7	2.9	17.6	-0.3
VI	22.0	26.0	4.0	25.0	3.0
Σ	87.3	115.7	28.4	96.3	9.0
Monthly precipitation sum (ΣR, mm)					
X	49.7	3.3	-46.4	0.0	-49.7
XI	40.0	62.7	22.7	22.1	-17.9
XII	49.7	58.6	8.9	134.0	84.3
I	45.9	52.2	6.3	27.0	-18.9
II	41.4	2.4	-39.0	26.6	-14.8
III	47.8	43.5	-4.3	60.3	12.5
IV	46.0	20.0	-26.0	64.0	18.0
V	53.1	78.4	25.3	68.9	15.8
VI	58.0	9.4	-48.6	11.0	-47.0
Σ	431.3	330.7	-100.6	413.5	-17.8

Variation analyses show that in the older varieties bred in the 1970s - Sadovo 1 (standard bread-making wheat, group B) and Pobeda (standard strong wheat, group A for 25 years) - the average grain yields are more strongly influenced by the type and amount of applied fertilizers and by agrometeorological conditions compared to the newly developed genotypes. In the varieties Avenyu and Magizhi (bred in 2011/2012), variation due to growing conditions is weaker, and consistently higher yields than the standard are obtained from the newest breeding lines included in the study (Table 2).

Table 2. Variation in grain yield (kg/da) by genotype

Genotype	Mean (M)	VC (%)	p-value
Sadovo 1	579	21	st
Pobeda	532	21	-
Avenyu	570	11	-
Magizhi	655	12	-
MH 258/3355	689	8	5%
RU 251/268	782	9	0.1%

Notes: VC = coefficient of variation. "st" indicates the standard (reference) variety. p-values reflect the significance level of differences from the standard.

Statistically significant differences in grain yield compared to the unfertilized control were obtained for the fertilization treatments with Lumbreko and for the conventional treatment with ammonium nitrate. In the Humeko variants,

the variation in grain yield increases due to agrometeorological conditions, and greater differences in genotype response are observed (Table 3).

Table 3. Variation in grain yield (kg/da) under different fertilization treatments

Fertilizer	Mean (M)	VC (%)	p-value
N 0	542	13	St.
N 12	722	10	1%
L 100	641	9	5%
L 200	674	12	5%
L 300	667	15	5%
L 400	697	12	5%
Hu 500	591	18	—
Hu 1000	612	28	—
Hu 1500	591	27	—

Notes: "St." indicates the standard (unfertilized control). L = Lumbreko; Hu = Humeko. N12 = ammonium nitrate at N12 equivalent.

The diagram (Figure 1) illustrates the individual response of each genotype to the applied soil improver.

Both fertilizers are 100% organic, but the uniform response and unidirectional effect of Lumbreko across all genotypes are likely due to the presence of auxins and gibberellins in its composition. Comparative studies indicate that both types of fertilizers (nanofertilizers and biofertilizers containing phytohormones) show potential to increase productivity, with varying degrees of effectiveness depending on specific conditions and varieties; levels of statistical significance vary with dosage and crop characteristics (Dhage & Biradar, 2020; Madlala et al., 2024; Kekeli et al., 2025).

The line RU 251/268 is the most strongly influenced by Humeko, and only in this genotype was a statistically significant difference in yield observed at all treatment levels (Table 4), showing a linear dependence on increasing fertilizer dose. In the remaining genotypes, a yield peak was recorded at a dose of 1000 ml/da, followed by a decline. The nanofertilizer Humeko contains Gram-positive bacteria, polysaccharides, and a wide range of microelements, whose effectiveness decreases with increasing dosage. According to available publications, the effect of nanofertilizers on wheat yield is stronger in cultivars that possess the following distinguishing characteristics: higher photosynthetic activity and biochemical mechanisms for nutrient uptake, greater sensitivity to controlled (precision) nutrient

delivery provided by nanofertilizers, rapid growth, early maturity, and improved technological quality (Garg et al., 2023; Ahmad et al., 2024). The line RU 251/268, due to its inherited high productive tillering capacity, large and

heavy grain, and more intensive growth, is able to utilize the higher levels of nutrient inputs. For the other genotypes, a more balanced nutrient supply using the tested nanofertilizer is recommended.

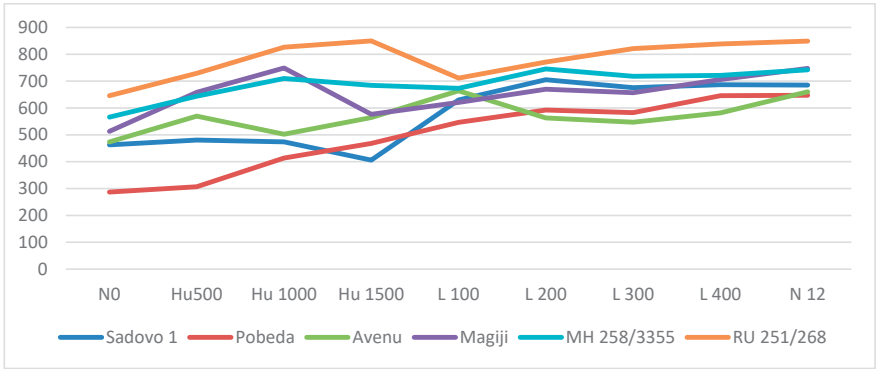


Figure 1. Response of the genotypes to fertilizer type and application rate

Table 4. Statistical significance of differences in grain yield, kg/da

Fertilizer	N 0	Hu500	Hu 1000	Hu 1500	L 100	L 200	L 300	L 400	N 12
Sadovo 1	463 st.	481 n.s.	474 n.s.	406 n.s.	630 n.s.	705 +	676 +	687 +	685 +
Pobeda	288 n.s.	307 n.s.	414 n.s.	468 n.s.	588 n.s.	547 n.s.	592 n.s.	583 n.s.	646 n.s.
Avenue	474 n.s.	570 n.s.	502 n.s.	564 n.s.	664 +	563 n.s.	547 n.s.	582 n.s.	660 +
Magizhi	513 n.s.	658 n.s.	749 +	576 n.s.	621 n.s.	670 +	657 +	706 +	747 ++
MH 258/3355	566 n.s.	644 n.s.	710 +	684 n.s.	673 +	745 ++	718 ++	722 ++	742 ++
RU 251/268	646 n.s.	729 +	826 ++	850 ++	711 +	771 ++	821 +++	839 +++	849 +++

Notes: n.s. = not significant; +, ++, +++ indicate levels of significance. “st.” = control standard.

Statistically significant difference in grain yield was observed between the two growing seasons (Table 5), while the variation attributable to genotype and fertilization was of intermediate magnitude.

Table 5. Variation in yields by genotype and fertilization

Year	Mean (M)	VC (%)	p-value
2024	550	18	-
2025	720	26	5%

The tests of between-group effects confirm the key role of agrometeorological conditions in modifying the influence of soil improvers on the productivity of wheat genotypes (Table 6). The individual effects of the studied factors are statistically significant across all variants of the experiment, but the combined effects of the

independent variables differ. The effect of Humeko is weaker, self-limiting at a level of 1000 ml/da, and the results of its application vary depending on the sink capacity of the genotype. The effect of Lumbreko depends primarily on the environmental conditions during the year; when sufficient moisture is available, the active ingredients of the fertilizer are effectively absorbed and lead to increased grain yield. As reported by other authors, nanofertilizers mainly enhance the delivery and efficiency of mineral nutrients, whereas biofertilizers containing phytohormones modulate growth and adaptation processes, creating a more complex effect on plants and providing more effective biological support for the development and productivity of cereal crops (Semykin et al., 2016; Yousefzadeh et al., 2021).

Table 6. Effect of factors on grain yield, kg/da

Tests of Between-Subjects Effects				
Source	Humeko		Lumbreko	
	Sig.	Effect	Sig.	Effect
genotype	0	22.3	0	9.7
dose	0	7.2	0	7.9
year	0	6.0	0	46.7
genotype *dose	0.051	8.4	0.772	3.0
genotype *year	0.628	0.7	0.033	1.9
dose *year	0.711	0.7	0	6.0
genotype *dose *year	0	24.0	0.917	2.4
Error	30.6		22.3	
Total	100		100	
$\alpha=0.05$	R Squared = 0.694		R Squared = 0.777	
	(Adj R Sq = 0.543)		(Adj R Sq = 0.666)	

CONCLUSIONS

In the older hybrid wheat breedings, grain yield is more strongly impacted by the type and quantity of applied fertilizers, as well as by agrometeorological conditions. The highest and most stable yields are obtained from the newest breeding lines MH 258/3355 and RU 251/268.

The effect of the phytohormone-based soil improver Lumbreko depends primarily on agrometeorological conditions, and when sufficient soil moisture is available, the active components of the fertilizer are efficiently absorbed. Increasing the dose leads to higher grain production, with yields at 400 ml/da being comparable to those under conventional fertilization.

The effect of the nanofertilizer Humeko on yield is weaker, limited at the 1000 ml/da level, and the results of its application depend on the sink capacity of the genotype. Only in the line RU 251/268 was a statistically significant difference in yield detected at all treatment levels and a linear response to increasing fertilizer dosage.

ACKNOWLEDGEMENTS

This research was funded by project BG16RFP002-1.014-0012-C01 “Establishment and sustainable development of a Center of Competence - Agrifood Systems and Bioeconomy”, financed by the European Regional Development Fund through the “Program for Research, Innovation and Digitalisation for Smart Transformation” (PRIDST).

REFERENCES

Ahmad, A., Akram, W., Sardar, R., & Yasin, N. A. (2024). Editorial: Interactive effects of plant growth-promoting microbes and nanoparticles on the

- physiology, growth, and yield of crops. *Front. Plant Sci.* 15:1338470. doi: 10.3389/fpls.2024.1338470
- Ahmadian, K., Jalilian, J., Pirzad, A. (2021). Nano-fertilizers improved drought tolerance in wheat under deficit irrigation. *Agricultural Water Management*, 244, <https://doi.org/10.1016/j.agwat.2020.106544>
- Ashurbekova, T., Omarova, P., Musinova, E., Efimov, D., Onkaev, V., & Krotova O. (2024). The expediency of using an integrated plant protection system and organomineral fertilizers in the conditions of the Southern natural and economic zone. *Bio Web of Conferences Interagromash* 113, 1-10.
- Dehodyuk, S. E., & Muliarchuk, A. O. (2023). Yield and grain quality of winter wheat under traditional and modern fertilization systems. *Agriculture and plant sciences: theory and practice.* 3(9), 37-42.
- Dhage, S.S., & Biradar, D. P. (2020). Nanofertilizers: Perspective to enhance growth, yield and NUE of crops, *Ind. J. Pure App. Biosci.* 8(6), 339-349.
- Elahi, M., Anas, M., Hayat, A. et al. (2025). Nitrogen Nano-fertilizers Enhance Wheat Productivity, Improve Nitrogen Use Efficiency, and Mitigate Soil Nitrogen Losses While Maintaining Grain Quality Compared to Conventional Fertilizers. *BioNanoSci.* 15, 477 <https://doi.org/10.1007/s12668-025-02065-4>
- Epanchintsev, V. Yu., & Ostrovskiy, K.V. (2020). Economic efficiency of improving the technology of growing spring wheat in western Siberia. *Economic sciences. Fundamental research*, 3, 32-37.
- Garg, D., Sridhar, K., Stephen Inbaraj, B., Chawla, P., Tripathi, M., & Sharma, M. (2023). Nano-Biofertilizer Formulations for Agriculture: A Systematic Review on Recent Advances and Prospective Applications. *Bioengineering*, 10, 1010.
- Gavrish, I. A., Lebedev, S. V., Korotkova, A. M., & Kvan, O. V. (2019). Effect of metal nanoparticles on physiological and biochemical parameters of soft Wheat. *Vestnik VGUIT [Proceedings of VSUET]*. 81(1). 263-268 (Ru). doi:10.20914/2310-1202-2019-1-263-268.
- Iagodzinska, A. (2020). Rational Use of Land Resources in the Production of Organic Products. *Modern Economics*, 22, 131-134.
- Kekeli, M. A., Wang, Q., & Rui, Y. (2025). The Role of Nano-Fertilizers in Sustainable Agriculture: Boosting Crop Yields and Enhancing Quality. *Plants.* 14, 554. <https://doi.org/10.3390/plants14040554>

- Le Campion, A., Oury, FX., Heumez, E. et al. (2020). Conventional versus organic farming systems: dissecting comparisons to improve cereal organic breeding strategies. *Org. Agr.* 10, 63-74 <https://doi.org/10.1007/s13165-019-00249-3>
- Madlala, N. C., Khanyile, N. & Masenya, A. (2024). Examining the Correlation between the Inorganic Nano-Fertilizer Physical Properties and Their Impact on Crop Performance and Nutrient Uptake Efficiency. *Nanomaterials*, 14, 1263.
- Novokhatsky, M., Targonya, V., Bondarenko, O., Lytovchenko, O., & Osipova, I. (2019). Development of technical and technological solutions for improving the efficiency of biological production. *Technical and technological aspects of development and testing of new machinery and technologies for agriculture in Ukraine*. 24(38), 236-243.
- Semykin, V. A., Pigorev, I. Y., Tarasov, A. A., Glinushkin, A. P., Plygun, S. A., & Sycheva, I. I. (2016). Microbial preparations and growth regulators as a means of biologization in agriculture. *RJOAS*, 11(59), 3-9.
- Sydiakina, O., & Dvoretzkyi, V. (2020). Productivity of winter wheat depending on food backgrounds in the conditions of Western Polissia. *Scientific Horizons*, 07(92), 45-52.
- Vinyukov, O., Chugrii, H., & Bondareva, O. (2023). Elements of technology as a means of obtaining stable and high gross yield of spring barley in the conditions of the steppe of Ukraine. *International scientific journal «Grail of Science»*, 31, 170-173.
- Volkova, L. V. (2023). The influence of hydrothermal conditions of the Kirov region on the productivity and quality of grain of spring soft wheat varieties of different ripeness groups. *Agricultural Science Euro-North-East*. 24(3), 377-388 (Ru).
- Yousefzadeh, S., Janmohammadi, M., & Sabaghnia, N. (2021). The effect of different nano-, bio- and conventional mineral fertilizers application on some morphological characteristics of maize. *Agriculture and Forestry*, 67(3): 135-145.
- Zastavnyy, Y. (2020). Organic agricultural production as a priority direction of development of rural households: financial and economic aspects. *Agrosvit*. 7, 125-132.